

P386-162427

Amphotericin B- and Folic Acid-Induced
Nephropathies in Developing Rats

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1985



U.S. Department of Commerce
National Technical Information Service

NTIS

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA/600/J-85/323	2.	3. PB 86-162427
4. TITLE AND SUBTITLE Amphotericin B-and Folic-Acid Induced Nephropathies in Developing Rats (Journal Version)	5. REPORT DATE 1985	
	6. PERFORMING ORGANIZATION CODE	
7. AUTHOR(S) R.J. Kavlock, B.F. Rehnberg, E.H. Rogers	8. PERFORMING ORGANIZATION REPORT NO.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS USEPA HERL, DBD, PTB Research Triangle Park, NC 27711	10. PROGRAM ELEMENT NO. ACSL1A	
	11. CONTRACT/GRANT NO.	
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development US Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED Journal Article	
	14. SPONSORING AGENCY CODE EPA-600/11	
15. SUPPLEMENTARY NOTES Toxicology and Applied Pharmacology 81, 407-415 (1985) December No. 3		
16. ABSTRACT The kidneys of newborn rats, which are both morphologically and physiologically immature, have been shown to be relatively insensitive to the nephrotoxic effects of several chemicals. To examine the specificity of these age-related differences, pups received either 20 mg/kg of Amphotericin B or 250 mg/kg folic acid, two known nephrotoxins in adult animals, on either postnatal day 1, 8 or 15. Renal function was examined by a basal clearance test and a hydropenia challenge at 1, 2 or 5 (6 in the case of Amphotericin B) days after treatment. We observed no difference in degree of renal toxicity with age, but repair of renal damage tended to proceed slower at the youngest age. Amphotericin treatment produced uremia, increased fractional excretion of water and sodium, a decreased fractional excretion of urea, and a diminished hydropenia response but no change in creatinine clearance and no renal pathology. The observed pattern of renal toxicity may be attributed to an inability to maintain urea gradients in the distal segment of the nephron. Folic acid treatment resulted in greatly increased kidney weights with marked pathology, uremia with decreased creatinine clearance, increased fractional excretion of water and a decreased hydropenia response. Unlike the Amphotericin effect, renal toxicity from folic acid appears to be a nonspecific response to cell death within the renal tubules. The data indicate that neonates do not possess a relative insensitivity to nephrotoxins in general and that renal physiological measurements which can be performed in neonatal rats are useful in evaluating and interpreting alterations in renal function.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED 20. SECURITY CLASS (This page) UNCLASSIFIED	21. NO. OF PAGES 10 22. PRICE

December
TOXICOLOGY AND APPLIED PHARMACOLOGY 81, 407-415 (1985)
No. 3

Amphotericin B- and Folic Acid-Induced Nephropathies in Developing Rats¹

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Received February 2, 1985; accepted July 22, 1985

Amphotericin B- and Folic Acid-Induced Nephropathies in Developing Rats. KAVLOCK, R. J., REHNBERG, B. F., AND ROGERS, E. H. (1985). *Toxicol. Appl. Pharmacol.* 81, 407-415. The kidneys of newborn rats, which are both morphologically and physiologically immature, have been shown to be relatively insensitive to the nephrotoxic effects of several chemicals. To examine the specificity of these age-related differences, pups received sc injections of either 20 mg/kg of amphotericin B or 250 mg/kg folic acid, two known nephrotoxins in adult animals, on postnatal Day 1, 8, or 15. Renal function was examined by a basal clearance test and a hydropenia challenge at 1, 2, or 5 (6 in the case of amphotericin B) days after treatment. We observed no difference in degree of renal toxicity with age, but repair of renal damage tended to proceed slower at the youngest age. Amphotericin treatment produced uremia, increased fractional excretion of water and sodium, a decreased fractional excretion of urea, and a diminished hydropenia response but no change in creatinine clearance and no renal pathology. The observed pattern of renal toxicity may be attributed to an inability to maintain urea gradients in the distal segment of the nephron. Folic acid treatment resulted in greatly increased kidney weights with marked pathology, uremia with decreased creatinine clearance, increased fractional excretion of water, and a decreased hydropenia response. Unlike the renal toxicity observed following amphotericin treatment, renal toxicity from folic acid appears to be a nonspecific response to cell injury within the renal tubules. The data indicate that, in general, neonates do not possess a relative insensitivity to nephrotoxins and that renal physiological measurements which can be performed in neonatal rats are useful in evaluating and interpreting alterations in renal function. © 1985 Academic Press, Inc.

The morphological immaturity of the kidneys in the neonatal period of many species including rabbits, dogs, and most rodents has been recognized since Kittleson (1917) described the formation of nephrons during postnatal growth in the rat almost 70 years ago. Since that observation, extensive work has been performed characterizing the functional aspects of renal maturation (Kleinman, 1978; Spitzer, 1978). Little attention has been paid,

however, to how this state of immaturity might influence the nephrotoxicity of chemicals.

One aspect of renal maturation known to influence toxicity of chemicals is the status of the organic ion transport systems. Cephaloridine, a broad spectrum antibiotic, induces proximal tubular damage in a variety of species, partially due to the activity of an anionic transport system which allows toxic amounts to accumulate within tubular cells. Rabbits are resistant to the nephrotoxicity of cephaloridine until they are 15 days old (Wold *et al.*, 1977), apparently the result of the poorly developed anionic transport system which precludes toxic concentrations from accumulating in the susceptible cells (Wold and Turnipseed, 1978).

¹ The paper has been reviewed by the Health Effects Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

For less well-defined reasons, metals also appear to be relatively ineffective in inducing renal toxicity in young animals. Mercuric chloride- (Kavlock, 1982; Daston *et al.*, 1983, 1984a) and mercurhydrin- (Wachstein and Robinson, 1965) treated neonatal rats and uranyl nitrate-treated puppies (Pelayo *et al.*, 1983) do not react with the characteristic signs of acute renal failure seen in similarly exposed adults. In addition, doses of sodium fluoride which induce renal toxicity in adults do not induce renal dysfunction in developing rats until they reach 29 days of age (Daston *et al.*, 1984b). However, no developmental pattern in the response of rabbits to mercuric chloride has been observed (Wold *et al.*, 1977), and it has been reported that acute renal failure can be induced in neonatal rats following treatment with the organomercurial diuretic mercaptomerin (Gray and Kavlock, 1983).

It was the intent of this study to further evaluate the development of sensitivity to nephrotoxic agents in rats by examining their response to two agents, amphotericin B and folic acid, which are different from one another in their mechanism of renal toxicity as well as from the aforementioned nephrotoxins.

METHODS

Animals. Groups of timed pregnant CD rats were obtained from Charles River Breeding Laboratories, Kingston, New York. In this strain, birth normally occurs on the evening of the 22nd day of gestation (Day 1 of gestation being the day that the presence of sperm in a vaginal smear is confirmed) and the following day is considered postnatal Day 1 (PD 1). On PD 1 all litters were reduced to 10 pups with as equal a sex ratio as possible and all pups were reared by their natural mother.

Treatment regimen. For each nephrotoxin, 30 such litters sharing the same birth date were established and divided into three subgroups of 10 litters each. Each subgroup was assigned for exposure to a nephrotoxin either on PD 1, 8, or 15. These ages were selected to span developmental periods ranging from early nephrogenesis (PD 1), to late nephrogenesis (PD 8), and finally, adult-like (PD 15) histological structure (Kittleson, 1917). Within each litter at each age, five pups each received either the vehicle or the freshly prepared nephrotoxin by sc injection in the nape of the neck. Injection volumes were 2 and 5 μ l/g and the vehicles were distilled water or 0.3 M NaHCO₃ (pH 6.5)

for amphotericin B and folic acid, respectively. Both nephrotoxins were obtained from Sigma Chemical Company. The doses of the nephrotoxins (20 mg/kg for amphotericin B and 250 mg/kg folic acid) were selected after consultation with the literature (Gouge and Andriole, 1971; Byrnes *et al.*, 1972, respectively) on effective doses in adult rats and after preliminary exposure of neonates to ensure that they were not overtly toxic.

Renal function measurements. At 1, 2, and 5 (folic acid) or 6 (amphotericin B) days after exposure to the nephrotoxins, the neonates were subjected to two tests of renal function: a basal clearance evaluation and a hydropenia challenge. These tests were selected to obtain a picture of how the glomeruli and tubules were functioning under normal physiological conditions as well as to examine how they functioned under a particularly demanding situation where failure of any one of many separate aspects of nephron operation would result in a compromised response. These time intervals were selected to encompass the periods of maximum expression of renal toxicity (the first 2 days) as well as a period when recovery of function following acute exposure is often observed. A pup was used for only one particular type of test, but as noted below, the pups used in the hydropenia challenge were reexamined on each day of the procedure.

At the beginning of the basal clearance test, the bladders were voided and 3 hr later urine was quantitatively collected by techniques described previously (Kavlock and Gray, 1982). This length of time was necessary for the pups to produce adequate urine volumes, yet minimized a hydropenic effect. Because rat pups are poikilothermic until approximately PD 12, they were placed in individual beakers containing about 5 cm of bedding and the beaker was placed in a water bath to keep the surface of the bedding at 32–34°C. After PD 12, animals were placed in small metabolism cages constructed of plastic beakers and funnels which drained to small vials in order to collect any spontaneous bladder voidings that were then possible. Due to the small size of the subjects, only a single blood sample could be obtained without seriously affecting overall blood volume. Blood samples were, therefore, collected only at the conclusion of the clearance period. Prior to PD 8, trunk blood was collected into Serum Separator Tubes (Becton-Dickenson) following decapitation; thereafter, blood was collected by external cardiac puncture under light CO₂ anesthesia (preliminary experiments in 8-day-old pups indicated no differences in the measured serum parameters due to this method of anesthesia versus collection of trunk blood following decapitation) and transferred to the separator tubes. Serum was obtained after centrifugation in an Eppendorf microfuge.

Aliquots of serum and urine were analyzed for sodium, potassium, urea, and creatinine in a Beckman ASTRA 4 Analyzer, chloride by a Radiometer CMT 10 Chloride Titrator, and osmolality with a Wescor Model 5100B Vapor Pressure Osmometer. Creatinine clearance (C_{cr}), and

fractional excretions (FE) of solutes were calculated by standard formulae.

The hydropenia challenge evaluates the ability of the neonate to elevate its urinary osmotic pressure in response to a period of fluid deprivation. In this procedure, pups were isolated from the nest for a total of 6 hr. Basal urinary osmotic pressure was determined from a urine sample collected at the beginning of the isolation period and compared with that obtained from a urine sample produced during the last 2 hr of the isolation period; urine volume was also determined for this period. Since this is a non-invasive technique, it was possible to return the pups to the nest at the conclusion of the challenge and reuse them on the other two examination days. Details of the experimental procedures and developmental profile of the hydropenia responses have been previously reported (Kavlock and Gray, 1982).

Pathology. At the conclusion of the clearance test, kidneys were removed, weighed, and preserved for histological examination in a solution of 5% formaldehyde, 5% acetic acid, and 70% ethanol. The kidneys were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by light microscopy by Experimental Pathology Laboratories (Raleigh, N.C.). All kidneys from the folic acid clearance animals were examined for pathology; however, because there were no gross changes in organ weight or coloration in the amphotericin B clearance animals, only a randomly selected fraction of them were evaluated for pathology, with the remainder held in reserve pending the preliminary outcome.

Statistics. Data were analyzed by the General Linear Models Procedure available in SAS (SAS, 1982) for the main effects of treatment and posttreatment interval, with interactions, for each nephrotoxin at each age period. In agreement with our historical data, preliminary data analysis did not indicate any biologically significant effects of sex on the measured variables, nor did it indicate any differential effects of toxin exposure on that related to sex of the individual; therefore, final data analysis did not include sex as a main effect in the model. Because the functional parameters in developing rat pups are undergoing rapid and considerable changes with age, direct comparisons between the magnitude of the effect at one age are difficult to make with those occurring at another age. Therefore, we chose to focus the statistical analysis on the time course of toxicant induced response and to make only qualitative comparisons across age. The magnitude of the differential age responses are shown in the figures, which depict the response of the pups as a percentage of the response of concurrent control pups. Note that the treatment induced effects are relatively consistent across ages when expressed in this manner, thus lessening the need to draw statistical comparisons across ages. When the analysis indicated a significant main effect ($p < 0.05$) or interaction ($p < 0.10$), individual means were compared by the least-squares procedure, with significances reported at the $p < 0.05$ level.

RESULTS

Amphotericin B

Basal clearance study. Administration of 20 mg/kg amphotericin B on PD 1, 8, or 15 had no effect on growth during the 6-day observation period (Figs. 1 and 2). A slight but consistent increase in relative kidney weight was noted in animals treated on PD 8. Although glomerular function appeared normal as evidenced by the clearance of creatinine, serum urea concentrations were elevated (range 32–115%) at all observation times at all ages in the treated groups. Occasional increases were noted for the fractional excretions of water, sodium, and potassium following treatment at the two older ages, while that of chloride was either reduced (the two youngest ages) or elevated (the oldest group) in amphotericin-treated animals. Despite elevated serum urea amounts, and hence, an increased filtered load, treated pups showed marked reductions in the fractional excretion of urea. The magnitude of these effects were generally the greatest 2 days after treatment, and tendency for return to control amounts was noted on the last day of observation.

Hydropenia challenge. Under basal physiological conditions, the osmotic pressure of the urine was unaffected by amphotericin (Fig. 3). However, treated pups were unable to respond to the need to conserve water when confronted with a 6-hr period of fluid deprivation, as evidenced by increased urine flow rates and decreased osmotic pressure compared to control values. This was particularly evident for those pups exposed at the two youngest ages, since urinary osmotic pressures barely exceeded the plasma osmotic pressure during the first 2 days after dosing.

Pathology. Kidneys from animals age PD 10 and younger showed the expected pattern of morphological immaturity and contained a thick zone of basophilic proliferating tubules beneath the renal capsule. A corresponding zone of loose embryonal connective tissue

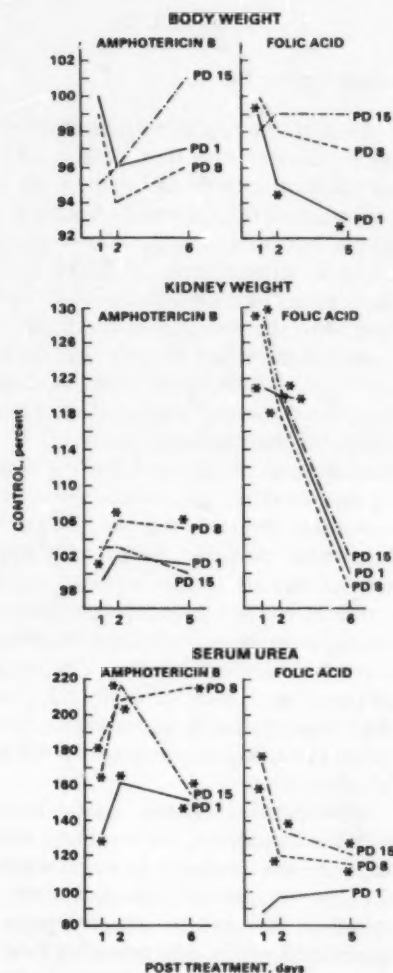


FIG. 1. Body weight, kidney weights, and serum urea concentrations in amphotericin B- and folic acid-treated pups as a function of age at time of exposure. To best present trends in response of treated pups, all values have been converted to percentage of concurrent control value. The original data were used in the statistical analyses. There were 10 pups in each dose group at each time point. An * denotes $p < 0.05$ significant difference from control value following 2-way ANOVA. Body weight ranged from 6.6 g in the youngest controls to near 59 g in the oldest. Relative kidney weights began between 11.5 and 11.8 mg/g in the youngest pups and reached 12.4 to 13.0 in the oldest. Serum urea concentrations were 10–12 mmol in the younger pups and declined to 6–7 mmol in the oldest control pups.

separated the renal tubules at the corticomedullary junction. In older pups, these zones had matured and were not observed microscopically. No treatment-related histopathologic changes or inhibition of maturation was noted in any treated group.

Folic Acid

Basal clearance study. Administration of 250 mg/kg folic acid to developing rat pups resulted in a growth retardation (3–7%) in the youngest age class; growth in older animals was unaffected (Figs. 1 and 2). Large increases (20–30%) in kidney/body wt ratios were evident with 24 hr of treatment. These kidneys showed marked yellowish discoloration which has been previously ascribed to deposition of folate crystals in the renal tubules. Relatively small increases in serum urea as well as decreases in creatinine clearance were observed occasionally. Changes in the reabsorptive ability for water, sodium, potassium, chloride, and urea were observed.

Hydropenia challenge. Under basal physiological conditions, pups exposed to folic acid on PD 1 appeared slightly dehydrated as evidenced by an elevated basal urinary osmotic pressure. Animals treated at older ages were not similarly affected (Fig. 3). Regardless of treatment age, urine flow rates were increased and osmotic pressure decreased during fluid deprivation, indicating a diminished ability of the kidney to respond to this stimulus. While the oldest treated animals had returned to control values by the last observation period, treatment-related effects were still evident in the two younger groups.

Pathology. Maturation changes noted in the amphotericin B study were again apparent in developing pups in the folic acid study. However, unlike the previous study, renal lesions were found in the treated groups (Table 1). These lesions resembled those previously reported for folic acid-treated rats and were characterized by eosinophilic casts and cellular debris in medullary tubules, scattered areas of dilated cortical tubules, and focal aggregates

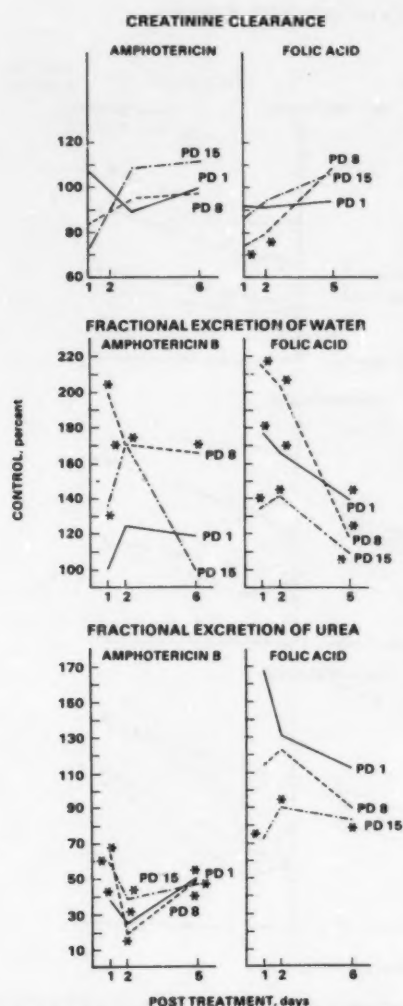


FIG. 2. Creatinine clearance and fractional excretions of water and urea in amphotericin- and folic acid-treated pups as a function of postnatal age at time of exposure. To best represent the trends in response of treated pups, all values have been converted to percentage of the concurrent control values. The original data were used in the statistical analyses. There were 10 pups in each dose at each time point. An * denotes $p < 0.05$ significance for control value following 2-way ANOVA. Creatinine clearances (in $\mu\text{l/g/min}$) were in the range of 0.9 to 1.0 in the youngest control pups and reached 2.8 to 3.8 in the oldest. Fractional excretions of water for the youngest control pups were in the range of 4.0%, and declined to 1.0 to 1.5% in the oldest. Fractional excretions of urea for the

of neutrophils around severely damaged tubules in the renal cortex and/or medulla. Lesions were most severe in pups killed 1 or 2 days after treatment; by 5 days after treatment they were minimal. In the immature kidneys of those rats treated on PD 1 or PD 8, medullary lesions were located in the inner medulla and not in the outer embryonal layer of the medulla. In those treated on PD 15, lesions were located in both the inner and outer medulla, but more casts were present in the distal tubules of the outer medulla. Cortical lesions were randomly distributed in immature kidneys, but were frequently located in the macula densa of the mature kidneys.

DISCUSSION

Amphotericin B is a polyene antibiotic produced by *Streptomyces nodosus* that is thought to disrupt membrane function in fungi by binding to ergosterol on the cell surface with resulting leakage of materials out of the cell. Specificity against fungi is attributed to the greater binding affinity for ergosterol than for cholesterol, the principal sterol of animals cell membranes (Medoff and Kobayashi, 1977). Renal toxicity, manifested by reduced creatinine clearance, tubular acidosis, and a vasopressin-resistant polyuria, is a recognized side effect of long-term amphotericin B treatment (Gouge and Andriole, 1971), primarily attributed to increased renal vascular resistance and tubular permeability to electrolytes (Cheng *et al.*, 1982). Acute exposure of rats to amphotericin results in minor histological alterations, primarily mild swelling of the glomeruli and proximal as well as distal tubules (Weldon and Schultz, 1974).

In the present study, acute exposure to amphotericin B proved to be an insidious nephrotoxin, affecting function in the absence of growth retardation or pathology, regardless of the age of the pup at treatment. The decreased

youngest control pups were in the range of 60–70% in the youngest controls and between 100 and 160% in the oldest.

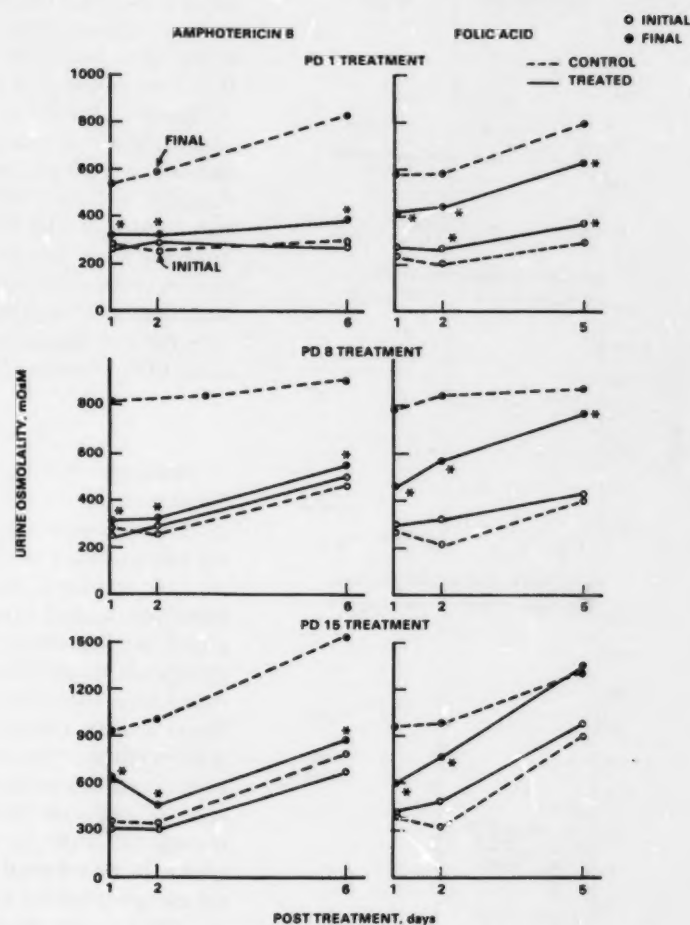


FIG. 3. Renal concentrating ability in amphotericin B- and folic acid-treated pups as a function of postnatal age at time of exposure. The initial urine osmolality value is obtained from an aliquot taken from the pups at time of removal from the nest. The final urine osmolality is that obtained during the last 2 hr of a 6-hr period of water deprivation. The sample size was 10 per dose per age per observation period. Standard errors for control pups ranged from 5.7 to 17.6% of the mean for initial osmolality and from 1.5 to 7.4% for final osmolality. An * indicates a $p < 0.05$ difference from concurrent control value. Note the almost total lack of concentrating ability in the amphotericin B-treated neonates and the ability of the folic acid-treated pups to approach control pups with increasing time after treatment (especially in the pups treated on PD 15).

fractional excretion of urea coupled with the increase in serum urea in treated animals was a particularly interesting observation. In the nephron, movement of urea is essentially governed by the reabsorption of water, which creates a concentration gradient, and the permeability of the tubular membrane, which

permits or blocks the diffusion of urea down its concentration gradient. The proximal tubule and descending limb of the loop of Henle are relatively permeable to urea, while the thick ascending limb, the distal tubule, and the collecting tubule are not. It is because of low permeability of these later segments of the

TABLE I
PATHOLOGY FINDINGS IN RATS EXPOSED TO FOLIC ACID DURING DEVELOPMENT^a

	Days after exposure					
	1		2		5	
	Control	Treated	Control	Treated	Control	Treated
Postnatal Day 1						
Casts in Medullary Tubules	0 ± 0 ^b	2.9 ± 0.2 (10) ^c	0 ± 0	2.5 ± 0.2 (9)	0 ± 0	0 ± 0
Dilatation of pelvis	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dilatation of medullary tubules	0 ± 0	3.1 ± 0.1 (10)	0 ± 0	2.7 ± 0.2 (9)	0 ± 0	0 ± 0
Dilatation of cortical tubules	0 ± 0	0.9 ± 0.1 (0)	0 ± 0	1.0 ± 0.2 (2)	0 ± 0	0.1 ± 0.1 (0)
Degeneration of cortical tubules	0 ± 0	0.5 ± 0.2 (0)	0 ± 0	0.5 ± 0.2 (0)	0 ± 0	0 ± 0
Degeneration of medullary tubules	0 ± 0	1.5 ± 0.2 (4)	0 ± 0	1.4 ± 0.2 (4)	0 ± 0	0 ± 0
Postnatal Day 8						
Casts in medullary tubules	0.1 ± 0.1 (0)	2.1 ± 0.1 (10)	0 ± 0	0.6 ± 0.3 (2)	0 ± 0	0 ± 0
Dilatation of pelvis	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dilatation of medullary tubules	0 ± 0	2.2 ± 0.1 (10)	0 ± 0	1.6 ± 0.3 (7)	0 ± 0	0.1 ± 0.1 (0)
Dilatation of cortical tubules	0 ± 0	1.1 ± 0.2 (2)	0 ± 0	0.9 ± 0.1 (0)	0 ± 0	0 ± 0
Degeneration of cortical tubules	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.2 (0)	0 ± 0	0 ± 0
Degeneration of medullary tubules	0 ± 0	0.8 ± 0.1 (0)	0 ± 0	0.1 ± 0.1 (0)	0 ± 0	0 ± 0
Postnatal Day 15						
Casts in medullary tubules	0 ± 0	1.4 ± 0.2 (5)	0 ± 0	1.0 ± 0	0 ± 0	0 ± 0
Dilatation of pelvis	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0
Dilatation of medullary tubules	0 ± 0	1.5 ± 0.2 (5)	0 ± 0	1.0 ± 0	0 ± 0	0 ± 0
Dilatation of cortical tubules	0 ± 0	1.1 ± 0.2 (2)	0 ± 0	0.8 ± 0.1	0 ± 0	0.7 ± 0.2 (0)
Degeneration of cortical tubules	0 ± 0	1.1 ± 0.2 (2)	0 ± 0	0.7 ± 0.2	0 ± 0	0.1 ± 0.1 (0)
Degeneration of medullary tubules	0 ± 0	0.8 ± 0.2 (1)	0 ± 0	0.3 ± 0.2	0 ± 0	0 ± 0

^a N = 10 per group, data presented as mean ± standard error.

^b Value is mean of subjective pathology score for all animals in a group for each particular lesion; 0 = normal, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe/high. Note severity of the medullary lesions in animals treated on PD 1; evidence for repair of damage is present in all groups.

^c Number in parenthesis is the number of kidneys examined which had a score of at least 2 for the lesion.

nephron that little filtered urea is reabsorbed back into the circulation. Three basic mechanisms exist whereby urea will accumulate in the blood: (1) an increase in urea production in excess of increases in its elimination; (2) a reduction in the filtered load caused by alterations in glomerular filtration rate (GFR) as is the case in acute renal failure; and (3) an increase in the permeability of the late nephron segments to urea. The first mechanism involves extra renal factors and is not associated with nephrotoxicity. In amphotericin-treated neonates GFR, estimated by the clearance of creatinine, was not lowered, thus the filtered load of urea did not appear to be changed. The third mechanism, therefore, appears likely to be responsible for the reduction in urea excretion and its increased presence in the serum.

This expression of toxicity is supported by the known pharmacologic effect of amphotericin that altered membrane permeability is due to binding to sterols and by the known effect of chronic amphotericin therapy in the production of a distal tubular acidosis (Gouge and Andriole, 1971). Inability to maintain urea gradients during passage of tubular fluid through the medulla could also account for the increased free water clearance and diminished hydropenia response that we saw in treated animals.

Regardless of the mechanism of the increased serum urea, the question arises as to the significance of such a change to the affected individual. Clearly, the affected individuals would have difficulty maintaining homeostasis during periods of restricted access to water,

but the effects of increased serum urea concentrations per se are difficult to evaluate. The precise role of urea in the pathogenesis of the uremic syndrome is open to much question, but the amounts of urea reached in the present study do not appear to be severely toxic (Kopple, 1983).

Administration of doses of folic acid has been shown to induce renal hypertrophy. This appears to be the result of partial tubular blockage caused by precipitation of the substance in the kidneys as a consequence of its low solubility under neutral or acidic conditions (Taylor *et al.*, 1968). Histologically, degeneration of tubular epithelial cells is observed in tubules that contain casts (folic acid) within 24 hr of injection of rats (Byrnes *et al.*, 1972; Schubert, 1976). Physiologically, folic acid-induced nephropathy is characterized by a vasopressin-resistant polyuria, decreased creatinine clearance, elevated fractional excretion of sodium, and glucosuria (Klinger *et al.*, 1980).

Folic acid-treated neonates exhibited nephrotoxicity, including morphological as well as physiological damage at all ages tested. Only mild effects on growth were observed, but large increases in relative kidney weights were seen. Histologically, damage was similar to that described for adult rats, although the site of the lesion was dependent upon the morphological maturity of the kidney. There was evidence that the lesions were being repaired, but functional deficits continued to be observed in the most immature animals 5 days after treatment. Unlike the results of the amphotericin B experiment, decrements in creatinine clearance accompanied increased serum urea amounts in the folic acid experiment, and there were no changes in the tubular handling of urea. Decreases in renal function, including alterations in water, sodium, and chloride conservation, are likely due to reductions in proximal tubular reabsorptive capacity resulting from tubular necrosis.

The principle objective of this study was to evaluate the age-related renal toxicity of two agents whose mechanisms of action differ substantially from other chemicals to which

young animals appear relatively insensitive. The data indicate: that neonates do not possess a generalized insensitivity to the action of nephrotoxins; that it is important to include both histological and physiological techniques in the evaluation of renal toxicity; that the methodologies employed in this study are capable of detecting perturbations in renal dysfunction in animals as young as 2 days of age; and finally, that it is important to evaluate a variety of physiological parameters in order to adequately describe and interpret alterations in renal function.

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